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Background

The first case ever of bluetongue (BT) in Denmark was recorded in October 2007, so the biting midge season in spring 2008, was awaited with some anxiety, due to the rapid spread of the BTV serotype 8 epidemic in the countries south of Denmark.

First outbreak in 2008

The first outbreak in Denmark 2008 was detected on August 27 in a cattle herd in Bredebro, which is located in the southern part of Denmark, approximately 20 km north of the German border. Initially two animals were suspected of having BT based on clinical symptoms so EDTA blood and serum samples were submitted to our laboratory. One of the animals tested positive by ELISA (OD% 15.52 1.4) and real time PCR[1] (Ct 27.1 0.4)

To evaluate the extent of this outbreak it was decided to test the remaining animals in pools from 5 samples.

Three pools were found positive for BT virus (BTV) by real time PCR. In each of these pools one sample of the five was positive for BTV RNA.

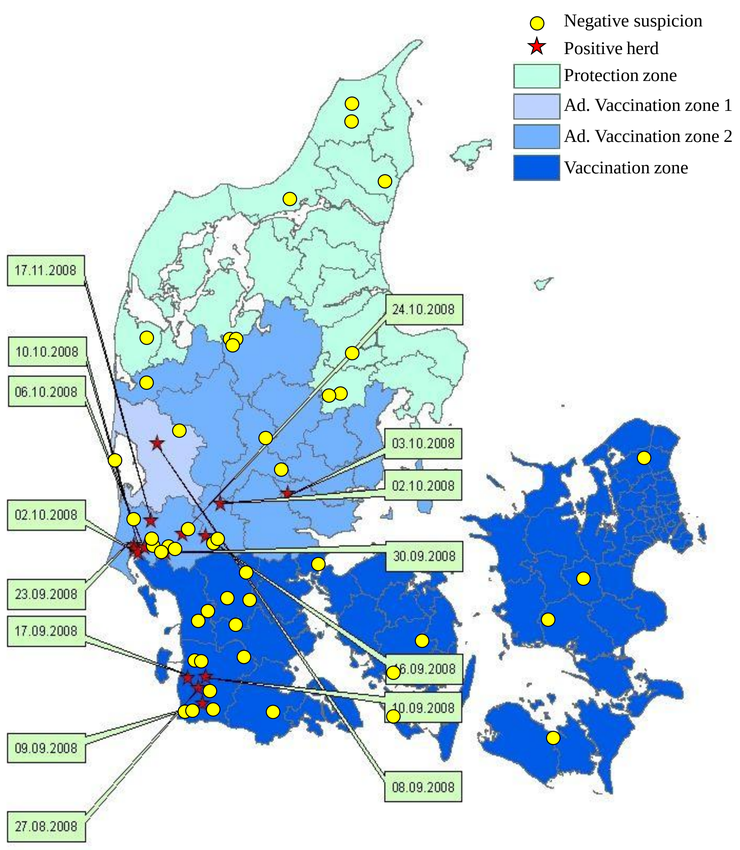
The animals were also tested individually for the presence of antibodies against BTV by ELISA.

Of the 75 animals tested, 35 were found positive for BTV specific antibodies. Of these 24 were cattle and the rest were sheep. The three PCR positive cows had high levels of anti-BTV antibodies (OD% 4.32 2.97) whereas the 21 antibody positive but PCR negative cows had lower levels of antibody (OD% 33.03 8.49).

The latter results are probably due to the fact that vaccination against BTV took place 9 days prior to the collection of blood and several animals had seroconverted in the intervening days.

	Antibodies ELISA		Real time RT-PCR Pool #	Real time RT-PCR Individual
Species	+/-	OD%	Ct	Ct
cattle	-			
cattle	+	15,496	1	
cattle	?	43,182	26,75	
cattle	+	3,375		24,53
cattle	+	39,738		
cattle	+	31,887	2	
cattle	-			
cattle	+	25,069		
cattle	+	39,187		
cattle	-			
cattle	+	1,928		28,52
cattle	?	46,006	3	
cattle	-		30,88	
cattle	-			
cattle	-			
cattle	-		4	
cattle	-			
cattle	-			
cattle	-			
cattle	-			
cattle	-			
cattle	+	38,636	5	
cattle	-			
cattle	-			
cattle	+	25,138		
cattle	+	7,645		23,04
cattle	-		6	
cattle	-		25,51	
cattle	-			
cattle	+	35,124		
cattle	-			
cattle	+	29,477	7	
cattle	+	24,862		
cattle	-			
cattle	+	30,441		
cattle	+	21,832	8	
cattle	-			
cattle	+	39,463		
cattle	-			
cattle	?	42,7		
cattle	?	43,802	9	
cattle	-			
cattle	+	36,295		
cattle	-			
cattle	+	24,931		
cattle	-		10	
cattle	-			
cattle	-			
cattle	+	35,606		
cattle	+	24,656	11	
cattle	-			
cattle	-			
cattle	-			
cattle	-			
cattle	-		12	
cattle	-			
cattle	-			
cattle	-			
cattle	-			
cattle	-		13	
cattle	-			
sheep	-			
sheep	+	4,614		
sheep	+	7,851		
sheep	-		14	
sheep	+	20,868		
sheep	+	32,094		
sheep	+	8,678		
sheep	+	14,738		
sheep	+	5,372	15	
sheep	?	45,248		
sheep	+	9,091		
sheep	+	3,237		
sheep	+	13,981		

Overview of Bluetongue in Denmark 2008



In total in Denmark during 2008 some 15 outbreaks of BT were registered. Out of approx. 65 clinical suspicions 11 were found positive and the remaining four outbreaks were discovered by the routine surveillance of bulk milk. All outbreaks were located in the south western part of the country but several outbreaks were north of the original vaccination zone resulting in two extensions of the vaccination zone during fall 2008. All outbreaks in Denmark during 2008 were caused by BTV-8.

From the first outbreak, a newly infected calf (still sero-negative) was brought to our animal facilities at Lindholm in order to be able to follow the development of anti-BTV antibodies and the level of viral RNA in the blood.

Blood was sampled frequently for a period of three months and analyzed by ELISA, real time PCR and virus isolation. The calf seroconverted one week after bluetongue was diagnosed and subsequently the level of antibodies increased for a month. Real time PCR values remained at an almost constant level throughout the entire three month period, Ct value at diagnosis was 22 and at slaughter the Ct was 25. Virus could be cultured in BHK cells until day 17 after diagnosis.

	Days after BTV diagnosis					
	0	7	17	27	34	81
Virus detection[a]	22	22	23	24	23	25
Antibodies[b]	neg	12,329	6,517	3,347	3,082	ND
Virus isolation[c]	+	+	+	-	-	-

a) Ct values of real time RT-PCR performed on RNA purified from EDTA stabilised blood

b) OD% of Elisa test performed on serum

c) Virus were cultured in BHK-21 cells from washed blood

Vaccination experiment

The clinical symptoms in the first herd were observed in connection with vaccination against BT and the blood, from the two first animals, submitted for examination was sampled approx. 30 min. after vaccination, which raised the question of whether the positive PCR results could be due to the vaccine.
In order to address this issue, a study was performed in which blood samples were collected at short time intervals immediately before vaccination until 96 h after vaccination (sampling times: 0.25, 1, 4, 24, 48 and 96 h).

Detection of Bluetongue virus and antibodies in newly vaccinated calfs

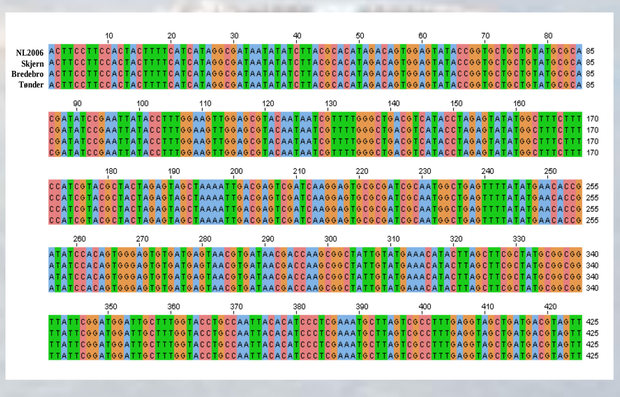
	Sampling time after Vaccination (h)						
	Real-time PCR						
Animal ID	0	0,25	1	4	24	48	96
4748							
4858							
4773							
4836			NO Ct				
4729							ND
4749							
	Antibodies						
4748							
4858							
4773							
4836			None				
4729							
4749							

Serotyping & sequencing

Bluetongue virus from all outbreaks were serotyped by real time PCR (Taqvet BTV8, LSI, Lissieu, France) and found to be BTV 8.

Sequencing of a part of segment 2 (using primer set $8W_1$[2]) was performed on BTV RNA from three outbreaks which were believed to be representative of all outbreaks. The most northern (Skjern, 08.09.2008) , the most southern (Tønder, 09.09.2008) and the first outbreak (Bredebro, 27.08.2008). See map under the dates for geographic location.

Sequences were identical from all three outbreaks and had 100% homology with the BTV8 isolates circulating in Holland in 2006.



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